Further Characterization of Dothistromin Genes in the Fungal Forest Pathogen *Dothistroma septosporum*

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ABSTRACT

Dothistroma septosporum is a forest pathogen that causes a disease called Dothistroma needle blight. The symptoms are thought to be due to the accumulation of dothistromin toxin produced by *D. septosporum*. Dothistromin is characterized as a difuranoanthraquinone and shows remarkable similarity to the aflatoxin (AF) and sterigmatocystin (ST) precursor versicolorin B. The similar structure to AF/ST suggests that dothistromin biosynthesis shares biosynthetic steps with the AF/ST pathway. The AF gene cluster in *Aspergillus parasiticus* and ST gene cluster in *A. nidulans* have been well characterized. Nine putative dothistromin biosynthetic genes have been identified. One of them, *dotA* was previously characterized by gene disruption and shown to have a similar function to homologous genes in AF/ST biosynthesis.

Two additional putative dothistromin biosynthetic genes, *pksA* and *epoA,* were characterized by gene disruption in this study. The inability of the *pksA* mutants to produce dothistromin indicated that the *pksA* is a key gene in dothistromin biosynthesis. The feeding of intermediates confirmed that *pksA* gene product is required for a very early step of dothistromin biosynthesis. The *pksA* mutants also showed reduced sporulation compared to wildtype, suggesting a relationship between dothistromin production and sporulation. The *epoA* gene replacements were also obtained successfully by homologous recombination. Both Southern blot and northern hybridization confirmed that the *epoA* gene was disrupted. However, the *epoA* mutants did not show any difference to the wild type in three analyses (growth rate, sporulation rate, dothistromin biosynthesis). However it was not possible to rule out a role for EpoA at a very late stage of dothistromin biosynthesis.

RACE analysis of the nine identified dothistromin genes characterized the transcription start and stop sites of the genes. Analyzing the putative regulatory protein binding motifs in the untranscribed region of the genes provided clues about the regulation of dothistromin biosynthesis and suggested there might be an *aflR*-like gene that governs dothistromin biosynthesis.

Both the *pksA* gene disruption and the RACE results suggested that the dothistromin biosynthetic pathway is homologous to that of AF/ST biosynthesis. Further work on the dothistromin gene cluster will help us to understand the evolution of fungal toxin gene clusters.

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Abbreviations

amp^r:	ampicillin resistance
bp:	base pair
cDNA:	complementary deoxyribonucleic acid
cm:	centimeter
°C:	degree celsius
CHEF:	contour-clamped homogeneous electric field
DNA:	deoxyribonucleic acid
dCTP:	deoxycytidine triphosphate
DEPC:	diethyl pyrocarbonate
DMSO:	dimethyl sulphoxide
DNase:	deoxyribonuclease
dNTP:	deoxynucleotide triphosphate
Fig:	figure
g :	gram
IPTG:	Isopropyl-β-d-thiogalactoside
kb:	kilobase pair
L:	litre
M:	mole per litre
ml:	milliliter
mM:	millimole per litre
OD_{600}	optical density at 600 nm
RNase:	ribonuclease
RNA:	ribonucleic acid
SDS:	sodium dodecyl sulfate
µl:	microlitre
µM:	micromole per litre
µg:	microgram
v/v:	volume per volume
w/v:	weight per volume
X-Gal:	5- bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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